STUDIES ON TISSUES OF FASTING ANIMALS.

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The changes rendered in the finer structure of tissues of fasting animals have been extensively studied and the results of these investigations have an important bearing upon our understanding of the inanition phenomena in general. Apart from the interest which the subject presents from a purely histological point of view, it throws light on many obscure problems regarding the transformation of materials within the organism occasioned by the fast.

The account here presented is based upon an examination of tissues from several dogs and one fox which had died of protracted inanition, having previously suffered a very large loss in body weight. These animals had been used in a number of metabolism experiments[1] conducted some years ago in the University of Illinois.[2] The tissues were removed immediately after the animal's death and fixed in Teleschnitzky's and Zenker's fluids. The material was carried through graduated alcohols and then preserved in eighty per cent. alcohol. It was embedded in paraffin, sectioned and stained in Delafield's hæmotoxylin, with eosin as a counterstain.

A superficial examination of the sectioned material, except in a few instances, reveals nothing abnormal. But a little attentive study is sufficient to appreciate the different ways in which the effect of prolonged inanition is stamped upon the histological elements of the organism.

Looking at the smooth muscles in the intestinal tract of every one of the animals which died of fasting the cells appear turbid and without a trace of longitudinal fibrillation. The

[1] Howe, Mattill and Hawk, *Jour. Biol. Chem.*, 10, 417, 1911 and 11, 103, 1912. Howe and Hawk, *Jour. Am. Chem. Soc.*, 33, 215, 1911; *Am. Jour. of Physiology*, 29, xiv., 1912, and 30, 174, 1912.

[2] We take this opportunity to acknowledge the material assistance which we received from the department of chemistry of the University of Illinois in defraying the expenses of the research.

fibers seem widely separated from each other, giving the entire muscle a very loose appearance. In cross section they are seen to consist of a dense central portion, which stains more or less strongly, surrounded by a colorless material. There is, however, no indication of a swelling of the muscle fibers as there is likewise no evidence of fatty degeneration, but they apparently undergo a process of liquefaction similar to that described by Miescher in the Rhein Salmon occasioned also by protracted fasting while it remains in fresh water. The nuclei are extremely irregular in outline and stain faintly.

In the voluntary, or striated muscle fibers, the cross markings lack the usual distinctness. Swelling or granular degeneration, such as described by Statkewitch, was never seen in our material.

Of all the organs of the body the liver is taxed most heavily during inanition inasmuch as it must take care of the products of metabolic activity of all other organs besides sustaining itself. It is natural to expect, therefore, to find the changes in the structure of the liver cells of a most pronounced character. Indeed, in the material under our examination a variety of degenerative phenomena has been observed. Considering the great difference in the degree of degeneration of the liver from animals which have all died of starvation it follows that death is not necessarily preceded by extreme cellular transformation. In our material every gradation from very slight changes to complete fatty degeneration of the liver cells could be observed. In two dogs which fasted 30 and 48 days respectively, whereby they lost 46 and 53 per cent. of their weight, there has been very little fatty degeneration in the liver. Some cells, however, were coarsely granular and others were riddled with vacuoles. In the case of the fox, which in 13 days of absolute fasting lost only 13 per cent. of its weight, the cells were found to be hollowed out by vacuoles of various sizes. These frequently encroach upon the nucleus and distort its shape as may be seen in Fig. 1. The vacuoles never show a very sharp outline, their boundary being more commonly diffuse and indefinite. In one extreme case of degeneration the liver presented complete transformation of its cells into typical fat cells. The polygonal shape of the cells was retained but the protoplasm was reduced to a mere band enclosing

a mass of fat. The cells seemed rather distended. The nuclei, pushed out to the periphery and usually into a recess of a corner, were flattened against the wall. Their staining capacity as well as that of the protoplasm was very feeble. The fatty degeneration was not equally intense in every portion of the liver, and here and there groups of intact liver cells could be seen whose poor staining power was the only evidence of degeneration.

No particular changes have been observed in nuclei. Cells with more than one nucleus are not uncommon, but these are found also in the normal liver. Phenomena of chromatolysis and vacuolization of the nuclei described by Statkewitch were never observed by us.

The histological structure of the stomach and intestine shows no striking changes. In sections of the stomach the oxyntic or parietal cells of the fundus glands are most conspicuous owing to their relatively large size and deep staining capacity. Their protoplasm is very granular. The other cells of the gland are small and their protoplasm is thin and practically colorless. The nuclei are usually normal, but in some portions, especially near the proximal end of the gland, they are much elongated and pressed against the cell wall adjoining the basal membrane. The two figures in the plate, 2 and 3, one a cross section of the upper region of the gland, the other a longitudinal section through the base of the gland, show these points. The clear, transparent character of the protoplasm is very well seen in the former, Fig. 2. The nuclei are always near the basal membrane.

The points brought out in the study of the fundus glands are also essential for all other glands as well as the mucous membrane of the intestine. The cells stain very feebly, their protoplasm being free of any granules. The nuclei migrate toward the basal membrane.

The phenomenon of particular interest, especially when viewed in the light of certain results of bacteriological studies on the permeability of the intestinal canal, is the invasion of the tissue underlying the mucous membrane as well as of the cells of the mucous membrane itself by numerous leucocytes. These occur not only singly but in groups of several cells together and occasionally accumulate in masses resembling solitary glands.

The submaxillary gland presents a few changes which are worth pointing out. The protoplasm of its cells, as was seen also in other gland cells, is thin in character and fails to take up the stain. Many cells are without nuclei, and the darkly stained crescent cells though present are generally flattened out and cap the outside of the alveoli like a narrow band. Fig. 4 shows that the submaxillary gland at the fatal termination of a protracted fast has all the appearance of a resting gland.

The most interesting set of modifications is to be observed in the kidneys. There one encounters various forms of degeneration and their distribution in the kidney is quite significant. We have already mentioned in discussing the changes in the liver that the extent of the degeneration of the histological elements apparently bears no relation to the death of the fasting animal as one of its direct causes. This statement holds equally true for the kidneys, where we found likewise a very wide range of modifications at the time of the animal's death.

The glomerulus has the usual lobulated structure but the Bowman capsule enclosing it is invariably thickened as in the case of nephritic kidneys. The cells of the convoluted tubules have a coarsely granular content and are invariably vacuolated. In some instances the vacuolization is so extensive as to give the tubule a striking honeycombed appearance. In Fig. 5 which is from a section of the kidney of the fox, this is shown very clearly. Similarly Fig. 6, which represents a section of a tubule in the kidney of a fasting dog, shows extensive vacuolization and the absence of boundaries between the cells. This last phenomenon, namely, the formation of a syncitium is characteristic not only of the kidney but also of the liver where the cells seem to melt together. In the ascending and descending limb of Henle's loop, however, vacuolization is a very rare occurrence, particularly in the later. The tubules were generally very granular in structure and contained frequently casts of various kinds, cellular, hyaline, etc. Fig. 5 is interesting furthermore on account of the well-preserved ciliated band lining the lumen of the tubule. The nuclei of the tubules are small and more or less irregular in shape. The cells of the collecting tubules show hardly any effect. The protoplasmic content is very clear

and free of granules. Here and there cells are found which have no nuclei. But when present the nuclei are relatively large and round, frequently bulging out into the lumen owing to the diminution of the cubical cells.

Before concluding this description of the changes which were observed in the tissues of fasting animals, a few words may be said concerning the condition of the testes and ovaries. In the former we failed to find any dividing cells. The nuclei were of the characteristic large round shape, whose chromatic content was intensely stained. It is noteworthy that in a very large proportion of the tubules the chromatic substance of all the nuclei was massed together to one side, as in the case of synizesis. It is hardly possible that this should be due to an artifact, as the nuclear condition varied in different tubules, but the former was found in most of them. The ovary which was examined seemed normal in every respect with numerous eggs in all stages of growth. We examined a number of fully developed eggs which were perfectly normal in every detail of their structure.

Bearing in mind that in inanition the organism is obliged to draw upon its own resources to derive the energy necessary for its maintenance, the metaplasmic material stored up in its cells and the depôts of fat are first to yield their quota to this stringent need. With the prolongation of the fast, as these reserve materials become reduced in quantity and at last disappear altogether, the substance of the cell body proper must contribute to the organism's demand for nourishment. It is now a well-established fact that various organs and tissues share unevenly in the support of the starving organism. As would be expected *a priori*, those elements of the organism the integrity of which is indispensable to its continued existence resist the pressure of the unfavorable conditions longest. This is true not only for the different systems of organs, but also for the minutest element of the organism, the cell, where the nucleus is usually the last part to fall prey to the exhausting effect of the fast. The nervous system likewise maintains its weight practically at a constant level as well as it preserves its morphological integrity until a very advanced stage in the fast.

Degenerative changes do not, as a rule, occur in any of the

tissues so long as the reserves of the body have not yet been entirely exhausted. The early appearance of fat globules in the liver of fasting animals led Mottram to believe that this process must be a physiological and not a pathological one. A similar opinion was likewise expressed earlier by Gilbert et Jomier. Mottram showed by means of histological examination of the liver of rabbits and guinea-pigs as well as by actual chemical investigation that with the advance of the fast an infiltration of the liver cells with fat from the depôts does take place. None of the authors who gave attention to this matter studied the liver of animals in very advanced stages of a fast. In our own case the animals succumbed after a loss of about 50 per cent. of their weight. There was very little histological evidence of a fat accumulation, but vacuolization of the cells was most prominent. It is hardly conceivable that the vacuoles were produced by the removal of the fat content by the reagents used in preservation in as much as it has been shown above that in one instance a liver was observed the cells of which in certain localities have undergone complete fatty degeneration. We are aware of the fact, of course, that a parallelism does not exist between histologically and chemically demonstrable fat in tissues. It may be that the infiltration of the liver with fat, especially in the early period of fasting, which is now proven beyond reasonable doubt (Mottram, Smirnow) and is very properly considered a physiological phenomenon, is concerned with the transfer of depôt fat to the rest of the tissues as food, while fatty degeneration of the liver cells, such as we observed over certain areas, is an independent phenomenon and is accompanied by the loss of the normal functional power of the cells. The view expressed here that the infiltration of the liver with fat may have to do with the conveying of the fat as nutriment to the starving tissues is borne out by Mottram's interesting observation on the qualitative change of the liver fat on different days of a short fast. There is a striking parallelism between the pure fatty acids present in the liver and the fat-quotient, *i. e.*, the ratio of the total fat of the liver to the initial body weight, showing that whenever an increase in fat content, *i. e.*, an infiltration, occurs it is due to an accumulation of fatty acids. If this view of the

rôle of the liver is correct it may also clear up the problem of the cause of the premortal rise in the nitrogen elimination. The latter was thought to be due either to an exhaustion of the entire supply of fat or to an excessive disintegration of cells. Neither the one nor the other of these hypotheses can be considered beyond criticism, because even in animals succumbing to a much protracted fast there is still sufficient fat present,[1] whereas there is no histological evidence of an unusual cellular destruction towards the end of the fast. A morphological and physiological degeneration of the liver interfering in some manner unknown to us with the endogenous fat metabolism probably results in an increased demand upon the body proteins which hastens the death of the animal.

In this connection it is interesting to point out that fat has never been demonstrated in tissues which in the fasting organism are among the strongest consumers, such as nervous and muscular tissues. The glandular tissues on the other hand, which are more or less deprived of their proper activity during a fast invariably show the presence of fat globules, according to Nicolaides. Later these fat globules disappear, leaving "empty spaces" which evidently correspond to our vacuoles. Nicolaides observed that in the gland cells of the duodenum and the pylorus small fat globules appear as soon as the animals commence to fast, whereby they invariably assume a regular arrangement in two parallel rows. We cannot agree with Nicolaides who considers the fat globules as "degenerative," and certainly see no reason for his assumption,—since their arrangement within the cell points against the supposition of a migration from fat depôts,—that they are formed from the protein constituents. The fact that in the submaxillary gland the fat globules appear only in the albuminous cells but never in the mucous or crescent cells, which has been observed by both Statkewitch and Nicolaides, cannot be taken as good proof of a formation of fat from protein. Also these facts become plain in the light of our hypothesis that the early appearance of the fat globules speaks decisively against any supposition that they result from degenerative transformation. We believe, on the contrary, that this is

[1] In the case of a dog which fasted 117 days and subsequently 104 days there were large masses of fatty tissue in the abdominal cavity at the time of death.

simply due to a qualitative transformation of the fat present in the cells whereby it becomes histologically visible. Since the staining reagents which are used in demonstrating the presence of fat globules are such that they react only with unsaturated fats, the appearance of the globules indicates that with the beginning of the fast a process of desaturation and probably the formation of fatty acids is started preliminary to the absorption of this fat to serve the nutritional needs of the organism.

The results of studies on fasting unicellular organisms where the conditions are simpler and easier to be appreciated support the view that vacuolization is one of the earliest and the most common degenerative process which ensues with the exhaustion of the reserves of the cell. Wallengren indeed in his most valuable research on inanition of infusoria distinguishes two periods, before and after the reserve material is exhausted. The former is accompanied by a gradual diminution of the animal, while in the second period the endoplasm becomes honey-combed with vacuoles of various sizes.

Vacuolization has been observed in various tissues: in ganglionic cells of the heart (Statkewitch), in the cells of the motor ganglia of the anterior horn (Schäffer) in bone marrow (Soltz), in the nephridial epithelium, etc.

Before concluding the paper mention should be made of another degenerative process which develops in the course of inanition. We refer to the gradual melting away of the cell boundaries which, with their complete disappearance, may even result in the formation of a syncytium. One of us described this condition in the liver of fasting salamanders. We also observed this phenomenon in our liver and kidney preparations. Similar observations have been made in fasting lower animals (Schultz). In the study of the salamanders it was shown how rapidly the cell walls are built up again around the intact nuclei as soon as the emaciated animals are once more given food.

At last the more or less universal loss of staining capacity due to the degenerative transformation produced by inanition and described by practically all authors must be pointed out.

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EXPLANATION OF FIGURES.

All figures have been made with the camera lucida and under the same magnification, using an objective No. 6 with an ocular piece No. 4 at normal tube length.

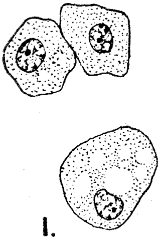
FIG. 1. A group of liver cells of a fasting fox.

FIGS. 2 AND 3. Cross-section and longitudinal section of fundus gland of a fasting dog.

FIG. 4. Section of submaxillary gland of fasting dog.

FIG. 5. Section of tubules in kidney of fasting fox.

FIGS. 6 AND 7. Section of convoluted tubule and of Henle's loop in kidney of fasting dog.



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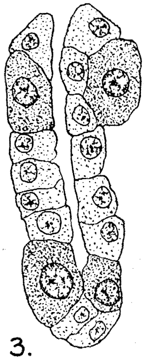

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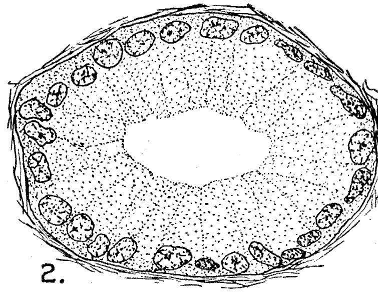

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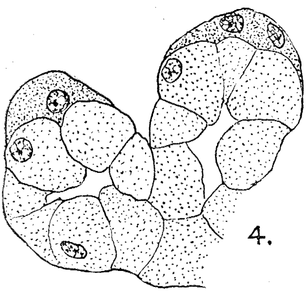

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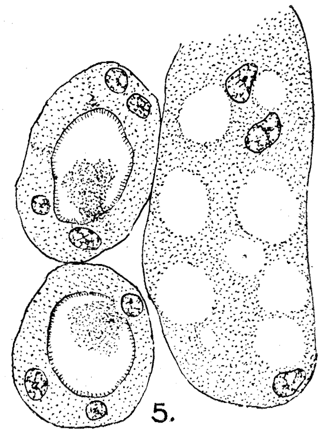

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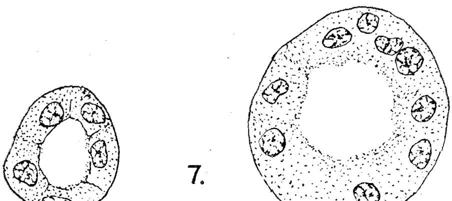

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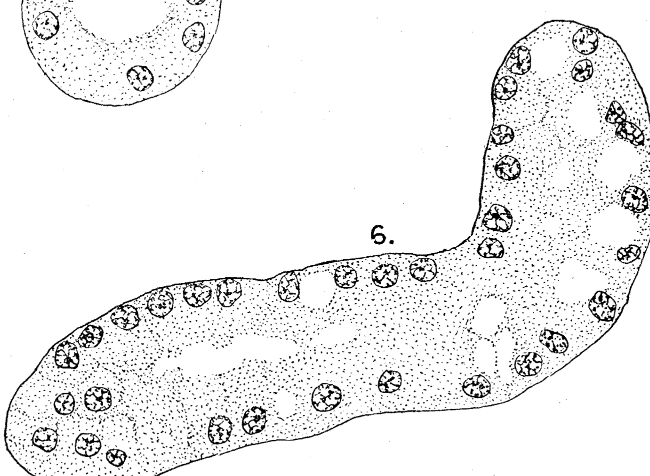

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